THE URINARY SEDIMENT
I love you, urine
THE URINARY SEDIMENT

- Introduction
- Main methodological aspects
- The particles of the urinary sediment of nephrological importance
- The urinary sediment in clinical practice
- Conclusions
INTRODUCTION
AN HISTORICAL PERSPECTIVE

• Introduced into clinical practice in Paris by François Rayer and Eugène N. Vigla in 1837

• By the end of the 19th century all the particles identified and the main urine profiles described

• A progressive decline in the 20th century, with the only exception, in the 1920s, for the contributions of Thomas Addis

• Some new interest after the paper in KI by Fairley and Birch on the utility of urinary red cell morphology evaluation by pha-co
• Mostly performed in central laboratories far from bedside and without the correct equipment and knowledge

• With the dream to entrust the whole test to automated instruments, which are already on the market (*UF100 and *iQ200*)

• Too often neglected even by nephrologists
MAIN METHODOLOGICAL ASPECTS
MAIN METHODOLOGICAL ASPECTS

* A correct urine collection

* A standardized method for the handling of urine

* A proper microscope

* A proper report
A CORRECT URINE COLLECTION

- Give the patient written and simple instructions:
  - Collect the first or the second urine of the morning
  - Avoid strenuous physical effort
  - Wash external genitalia
  - Male: uncover the glans
  - Female: spread the labia of vagina
  - Collect mid-stream urine

- Avoid urine collection during menstruation

- Give the patient a proper urine container
A STANDARDIZED METHOD FOR THE HANDLING OF URINE

- Second urine of the morning produced over a period of two hours
- Centrifugation of a 10 ml aliquot of urine for 10 min at 400g.
- Removal of 9.5 ml of supernatant urine
- Gentle but thorough resuspension by pipette of the sediment in the remaining 0.5 ml of urine
- Transfer by pipette of 50 µl of resuspended urine to a glass slide covered with 32 x 24 mm coverslip
- Examination of the samples at low (160x) and high (400x) magnification within 3h from collection

Particles expressed as lowest–highest number/microscopic field

NB. Avoid “pouring off” procedures and delays in handling the samples
A PROPER MICROSCOPE

* Of good quality
* Low magnification (eg, 160x)
* High magnification (eg, 400x)
* Phase contrast
* Polarized light
URIC ACID - PHASE CONTRAST
LIPID DROPLETS - PHASE CONTRAST
LIPID DROPLETS - POLARIZED LIGHT
A URINARY SEDIMENT REPORT

DATE ....................................................................................................................
SURNAME .................................................. NAME ..............................................................................

pH ........ DENSITY ...............HAEMOGLOBIN: ..........LEUKOCYTE ESTERASE ...........

ERYTHROCYTES: .................
ISOMORPHIC (%) ..............DYSMORPHIC (%) .................... ACANTHOCYTES (%)..................
LEUKOCYTES: ..............................................................
TUBULAR CELLS: ..............................................................
TRANSITIONAL CELLS: SUPERFICIAL ................................ DEEP...........................
SQUAMOUS CELLS: ..........................................................................................
CASTS: ....................................................................................TYPES: ..........................................................

LIPIDS: ..............................................................
CRYSTALS: ..............................................................
BACTERIA: .............................................................. YEASTS: ....................................................
OTHERS: ..........................................................................................

COMMENT ..............................................................................................................

..........................................................................................................................

SIGNATURE
...........................................................................................
A URINARY SEDIMENT REPORT

DATE .............................................................................................................
SURNAME .......................................................................................... NAME ..............................................................
pH ........6.0....... DENSITY .......1.006...... HAEMOGLOBIN: ........+++..... LEUKOCYTE ESTERASE ....+++.....

ERYTHROCYTES: ........1–3/HPF.......... ISOMORPHIC (%) ........ DYSMORPHIC (%) ................ ACANTHOCYTES (%) ........
LEUKOCYTES: ........1–2/HPF ..............................................................................................................................................
TUBULAR CELLS: ..................................// ..........................................................................................................................
TRANSITIONAL CELLS: SUPERFICIAL: ......................//.................. DEEP: .................................. SQUAMOUS CELLS: ......./........................//.......................................................... TYPES: ..........................................................

 CASTS: ........................................//.......................................................... LIPIDS: ........................................//..............................
CRYSTALS: ........................................//.......................................................... BACTERIA: ........................................//......................... YEASTS: ........................................//..............................
OTHERS: ........................................//..........................................................

COMMENT. MILD ERYTHROCYTURIA AND LEUKOCYTURIA. PLEASE NOTE THE DISCREPANCY BETWEEN DIPSTICKS FOR HAEMOGLOBIN AND LEUKOCYTE ESTERASE AND MICROSCOPY. THIS IS PROBABLY DUE TO CELL LYSIS CAUSED BY LOW DENSITY.

SIGNATURE .....................................................................................
THE PARTICLES OF THE URINARY SEDIMENT OF NEPHROLOGICAL IMPORTANCE
PARTICLES OF NEPHROLOGICAL IMPORTANCE

- Cells
- Lipids
- Casts
- Crystals
- Microorganisms
Cells

From Blood

- Erythrocytes
  - Type: Isomorphic
  - Subtype: Dysmorphic
- Leukocytes
  - Type: Neutrophil
  - Subtype: Eosinophil, Lymphocyte
- Macrophages
  - Type: Granular
  - Subtype: Homogeneous, Phagocytic

Epithelial

- Transitional Cells
  - Type: Proximal
  - Subtype: Distal
- Squamous Cells
  - Type: Superficial
  - Subtype: Deep
ERYTHROCYTES

• A frequent finding (53% of samples)

• Two main types: glomerular and non-glomerular
GLOMERULAR OR DYSMORPHIC ERYTHROCYTES
NON GLOMERULAR OR ISOMORPHIC ERYTHROCYTES
Hematuria: A simple method for identifying glomerular bleeding

Kidney Int 1982; 21: 105-08
DETECTION
OF GLOMERULAR BLEEDING BY PHASE-CONTRAST MICROSCOPY

> 80% cut-off for
the definition of a haematuria as
glomerular or non glomerular

Lancet 1982; I: 1432-34
PROBLEMS ASSOCIATED WITH THE ANALYSIS OF U-RBC MORPHOLOGY

➢ Requires experience

➢ Is exposed to the risk of low inter-observer reproducibility

➢ Still lacks of univocal criteria for defining a haematuria as glomerular or non glomerular
Köhler H, Wandel E, Brunck B

Acanthocyturia-A characteristic marker for glomerular bleeding

ACANTHOCYTE OR G1 CELL (SEM)
ACANTHOCYTES OR G1 CELLS (PH-CO)
DIAGRAM OF THE COMMONEST TYPES OF ACANTHOCYTES OR G1 CELLS
<table>
<thead>
<tr>
<th>Types</th>
<th>More often PMNs (but also eosinophils and lymphocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>For PMNs, any segment of the urinary tract (without forgetting genital contamination)</td>
</tr>
<tr>
<td>Clinical meaning</td>
<td>Inflammation of whatever cause including immunological disorders (eg, glomerular diseases)</td>
</tr>
</tbody>
</table>
POLYMORPHONUCLEAR LEUKOCYTES
# Diseases Associated with Eosinophiluria

<table>
<thead>
<tr>
<th>Disease</th>
<th>N</th>
<th>Hansel</th>
<th>Wright</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>11</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>RPGN</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Postinfectious GN</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ATN</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute pyelonephritis</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute prostatitis</td>
<td>10</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

LYMPHOCYTES (by PH-CO)
LYMPHOCYTURIA

- An early marker of acute cellular rejection of renal allograft
- Sensitivity 80 - 90%
- Stains and cytological techniques needed
TUBULAR CELLS

Different morphological subtypes from different tubular segments
PROXIMAL TUBULAR CELL
DISTAL TUBULAR CELL
COLLECTING DUCT CELL
TUBULAR CELLS

Clinical meaning

- Acute tubular necrosis
- Acute interstitial nephritis
- Glomerular diseases (especially proliferative types)
# URINARY LIPIDS

| **Appearance** | Fatty droplets  
|               | “Oval fat bodies”  
|               | Fatty casts  
|               | Cholesterol crystals |
| **Source**    | Lipid ultrafiltration due to abnormal GBM permeability |
LIPID DROPLETS
INTRACELLULAR LIPIDS
“OVAl FAT BODY”
FATTY CAST
URINARY LIPIDS

Clinical meaning

- Marked proteinuria

- Lipid storage diseases
  (eg, Fabry disease)
## CASTS

<table>
<thead>
<tr>
<th>Formation</th>
<th>Distal tubules and collecting ducts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix</strong></td>
<td>Tamm-Horsfall glycoprotein</td>
</tr>
<tr>
<td><strong>Different types</strong></td>
<td>Different clinical meanings</td>
</tr>
</tbody>
</table>
ERYTHROCYTE CAST
LEUKOCYTE CAST
BACTERIAL CAST
YEAST CAST
URINARY CRYSTALS

• “COMMON” CRYSTALS

• PATHOLOGICAL CRYSTALS

• CRYSTALS DUE TO DRUGS
URIC ACID (U-pH ≤ 5.4)
CALCIUM OXALATE MONOHYDRATED (U-pH <5.4-6.7)
CALCIUM OXALATE BY HYDRATED (U-pH < 5.4-6.7)
CALCIUM PHOSPHATE (U-pH ≥7.0)
TRIPLE PHOSPHATE (U-pH ≥7.0)
PATHOLOGICAL CRYSTALS

- CHOLESTEROL
- CYSTINE
- LEUCINE
- TYROSINE
- 2,8-DI-HYDROXYADENINE
CHOLESTEROL
2,8-HYDROXYADENINE (BF)
2,8-HYDROXYADENINE (POL)
Adenin phosphoribosyltransferase (APRT) deficiency

APRT

Adenine \rightarrow Adenosine monophosphate

XANTINE OXIDASE

2,8-dihydroxyadenine (2,8-DHA) (highly insoluble at any pH)
APRT DEFICIENCY

Inherited in an autosomal recessive manner

**Type I APRT deficiency:** enzyme activity in erythrocyte lysate: virtually absent
Affected patients: whites, homozygotes or compound heterozygotes

**Type II APRT deficiency:** residual enzyme activity in erythrocyte lysate: 10-25%
Affected patients: Japanese, mostly homozygotes
CLINICAL FEATURES

¬ M/F 12/11 Age 0.5-62 yrs (m ± SD: 29.0 ± 19.8)

¬ Recurrent radiolucent stone disease (15/23: 65%)

¬ ARF due to intratubular 2,8-DHA precipitation (6/23: 26%)

¬ CRF due to ? chronic interstitial nephritis (4/23: 17%)

¬ 2,8-DHA crystalluria (22/23: 96%)
ARF FROM INTRATUBULAR PRECIPITATION OF 2,8-DHA
ARF FROM INTRATUBULAR PRECIPITATION OF 2,8-DHA
DIAGNOSIS

• Level of residual enzyme activity in erythrocyte liseate

• Measurement by HPLC of urine 2,8-DHA

• UV and infrared spectrophotometry of stones (biochemical analysis does not differentiate 2,8-DHA from uric acid)

• Urine sediment examination
### Main Types of Crystals Due to Drugs (I)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Crystals</th>
<th>Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphadiazine</td>
<td>Birefringent “shocks” of wheat or “shells” with striations</td>
<td>Isolated crystalluria, haematuria, ARF, Stones</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Birefringent fine needles</td>
<td>Isolated crystalluria? ARF</td>
</tr>
<tr>
<td>Indinavir</td>
<td>Plate-like rectangles, star-like forms, irregular plates, strongly birefringent</td>
<td>Isolated crystalluria, stones, ARF</td>
</tr>
<tr>
<td>Piridoxylate</td>
<td>Asymmetrical hexagons of rectangles with rounded extremities</td>
<td>Stones</td>
</tr>
</tbody>
</table>
# Main Types of Crystals Due to Drugs (II)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Crystal</th>
<th>Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primidone</td>
<td>Birefringent hexagons</td>
<td>Isolated crystalluria, transient proteinuria and haematuria</td>
</tr>
<tr>
<td>Naftidrofuryl Oxalate</td>
<td>Birefringent monohydrate calcium oxalate</td>
<td>ARF</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Birefringent monohydrate calcium oxalate</td>
<td>Acute renal failure</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>Needles Shocks of wheat</td>
<td>Isolated crystalluria Haematuria ARF</td>
</tr>
</tbody>
</table>
AMOXYCILLIN (BF)
ACYCLOVIR
FACTORS FAVOURING DRUG CRYSTALLURIA

- Drug overdose
- Dehydration
- Hypoalbuminaemia
- Urine pH
CLINICAL MANIFESTATIONS OF DRUG CRYSTALLURIA

- Isolated and asymptomatic crystalluria
- Haematuria (micro or gross) ± leukocyturia
- Obstructive uropathy due to drug stones
- ARF due to intratubular precipitation of crystals
GENERAL RULES TO RETAIN ABOUT CRYSTALLURIA DUE TO DRUGS

1) Think of a drug whenever you come across crystals with unusual appearance

2) Ask the patient *if* and *which* drug is taking

3) Check the renal function

4) Hydration of the patient and reduction/discontinuation of the drug to prevent ARF
# MICROORGANISMS

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>Rods and cocci</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEASTS</td>
<td>Candida</td>
</tr>
<tr>
<td>PROTOZOA</td>
<td>Trichomonas vaginalis</td>
</tr>
<tr>
<td>PARASITES</td>
<td>Enterobius vermicularis</td>
</tr>
<tr>
<td></td>
<td>Schistosoma haematobium</td>
</tr>
</tbody>
</table>
URINARY SCHISTOSOMIASIS

- Endemic in many geographic areas
- Responsible for:
  - recurrent bouts of gross haematuria "male menstruation"
  - obstructive uropathy
  - bladder carcinoma
  - glomerulonephritis
DIAGNOSIS OF URINARY SCHISTOSOMIASIS

- It is largely based on the examination of the urinary sediment
- To increase the egg yield (and hence sensitivity) the US is examined after a physical effort (eg, a run) and between 10 am and 2 pm
- The quantitation of the eggs is used to estimate the severity of the infection
EGG OF SCHISTOSOMA HAEMATOBIUM (120-150 μm)
THE URINE PROFILE OF URINARY SCHISTOSOMIASIS

- Associated findings in 50 subjects*

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>100%</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>92%</td>
</tr>
<tr>
<td>Uroepithel cells</td>
<td>28%</td>
</tr>
<tr>
<td>Albumin (2+/3+)</td>
<td>14%</td>
</tr>
</tbody>
</table>

*Hôpital St. Jean De Dieu, Tanguïéta, Republic of Benin
THE URINARY SEDIMENT IN CLINICAL PRACTICE
THE URINARY SEDIMENT IN PERSISTENT ISOLATED MICROSCOPIC HAEMATURIA (PIMH)
HOW WE CLASSIFY THE HAEMATURIA

For each sample:

• We first evaluate the morphology of 100 RBCs and then give the percent of isomorphonc and dysmorphic RBCs (including acanthocytes)

• We consider a haematuria as glomerular when:
  • Acanthocytes are $\geq$ 5% or
  • A mixed pattern is found (dysmorphic RBCs $\geq$ 40%) or
  • Dysomorphic RBCs are $>$ 80%

• We also look for erythrocyte casts (N/50 LPFs)
Between 1994 and 2003, we have identified 15 patients (10 children and 5 adults) whose PIMH was defined as “glomerular” after the examination of repeated urinary samples (2-8 patient; total number 52).

All these patients were submitted to renal biopsy.
THE URINARY SEDIMENT IN PROLIFERATIVE AND NON PROLIFERATIVE GLOMERULAR DISEASES
TRANSPLANTED KIDNEY

• Recurrence of a glomerular disease:
  Dysmorphic RBCs ± RBC casts

• Acute cellular rejection:
  Lymphocyturia ± tubular cells

• Urinary tract infection:
  WBCs + bacteria ± WBC/TC casts

• Infection due to polyomavirus BK ± BKVN:
  Decoy cells
POLYOMAVIRUS BK NEPHROPATHY (BKVN)
BK VIRUSN NEPHROPATHY

- A disease of the transplanted kidney due to the invasion of the renal parenchyma by PVBK

- At RB, ATN, interstitial cellular infiltrate ± tubulitis and typical tubular cytopathic changes
BKVN: CLINICAL FEATURES

- Progressive deterioration of renal function in patients treated with new immunosuppressive agents (especially tacrolimus and/or MMF)
- Prevalence: \( \sim 4.9\% \)
- Graft loss: 46% of patients
- Treatment: ↓ of immunosuppression
DIAGNOSIS

• Renal biopsy
• Urinary cytology
• Measurement of BKV-DNA in blood and urine (by real time PCR)
NUCLEAR CHANGES DUE TO BKV AS SEEN BY PHASE CONTRAST

- Enlarged nucleus ("ground glass" appearance)
- "Margination" of chromatin
- Irregular chromatin pattern
- Multiple nuclear inclusion bodies of different size and shape
- Unique and large nuclear inclusion body with a clear perinuclear halo ("bird’s eye" appearance)
- Vacuolated nucleus (very rare)
- Cytoplasmic vacuoles (common)
DECOY CELLS BY PHASE CONTRAST
DECOY CELLS BY PAPANICOLAOU
URINALYSIS IN BKVN

- Proteinuria: 2/8 ($\leq 0.5g/24h$)
- Decoy cells: 8/8
- RBC/Haemogl: 0/8
- WBCs/Leuk est: 0/8
- Uroepith cells: 1/8 (1+)
- Casts: 1/8 (1+)
- Lipids: 0/8
- Macrophages: 4/5
URINARY MACROPHAGES
GRANULAR MACROPHAGE
VACUOLAR MACROPHAGE
PHAGOCYTIC MACROPHAGE
CONCLUSIONS
CONCLUSIONS (I)

- The examination of the urine sediment is the most inexpensive and quick test in our hands.
- It is also one of the few tests which can be performed at bedside.
- When performed with the correct methodology and knowledge, it can be useful in a wide range of renal diseases.
“So great it is the potentiality of the examination of the urine sediment that it should be carried out by the physician himself… The two to five minutes of additional time consumed often will richly be rewarded”

G.E. Schreiner 1961
CONCLUSIONS (III)

Why, then, such a valuable test is so frequently neglected by us nephrologists?